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FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
PP01521.101	Unknown 09/762693

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/18087	10 August 1999	8 August 1998

TITLE OF INVENTION

ENGINEERED ANTIGEN-PRESENTING CELLS EXPRESSING AN ARRAY OF ANTIGENS AND USES THEREOF

APPLICANT(S) FOR DO/EO/US

Lewis T. WILLIAMS, Martin GIEDLIN, Jaime ESCOBEDO, Amy L. COLLINS and Timothy FONG

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Copy of Published Specification
Written Opinion

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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/762693 Unknown	PCT/US99/18087	PP01521.101

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$1,860.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	40 - 20 =	20	x $18.00	**$360.00**
Independent claims	2 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$270.00**
	TOTAL OF ABOVE CALCULATIONS =			**$2,490.00**
☐	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			**$0.00**
	SUBTOTAL =			**$2,490.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$130.00**
	TOTAL NATIONAL FEE =			**$2,620.00**
☐	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			**$0.00**
	TOTAL FEES ENCLOSED =			**$2,620.00**
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$2,620.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **03-1664** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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31,261
REGISTRATION NUMBER

9 February 2001
DATE

1/PRTS

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ENGINEERED ANTIGEN-PRESENTING CELLS EXPRESSING AN ARRAY OF ANTIGENS AND USES THEREOF

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

TECHNICAL FIELD OF THE INVENTION

The invention relates to immunotherapy involving the activation of T lymphocytes by antigen-presenting cells that are genetically modified to express and present on their surface an array of antigens that are differentially expressed by a target population. The antigen-presenting cells can be genetically modified by transduction with nucleic acid sequences identified by differential screening of nucleic acid sequences expressed in a target population relative to a non-target population.

BACKGROUND OF THE INVENTION

Dendritic cells, which are specialized antigen-presenting cells, can be used to process antigens from diseased tissue and present them to the immune system. Dendritic cells are leukocytes derived from bone marrow and are considerably more potent than other antigen-presenting cells with respect to presentation and activation of cytotoxic T lymphocytes (CTLs). Publications relevant to dendritic cells include Young et al. 1996, J. Exp. Med. 183:7-11; Alavaikko et al. 1994, Am. J. Clin. Pathol. 101:761-767; Shunichi et al. 1995, Cancer 75:1478-1483; Hsu et al., 1996, Nature Medicine 2:52-58; Mayordomo et al. 1995, Nature Medicine 1:1297-1302; Paglia et al. 1996, J. Exp. Med. 183:317-322; and Boczkowski et al. 1996, J. Exp. Med. 184:465-472.

There is a need for a vaccine that can boost multiple clones of CTLs to enhance the immune response and to prevent the escape of a tumor from immune selection.

Accordingly, there is a need for a system for providing antigen-presenting cells with (i) a broader spectrum of potential anti-tumor antigens and (ii) providing the antigens in a form which the antigen-presenting cells can effectively process and present.

SUMMARY OF THE INVENTION

The invention provides a system for priming antigen-presenting cells with a repertoire of antigens of a specific cell type, for example, a tumor cell or a virally-infected cell. Further, the approach need not be restricted to stimulating an immune response against diseased tissue. It can be used to mount an immune response against any target tissue and to ablate any target cell expressing a particular set of antigens.

The invention provides a method of producing at least one vector encoding an array of antigens for expression in an antigen-presenting cell. In one embodiment, the method comprises comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population. The method further comprises selecting nucleic acid sequences preferentially expressed by the target cell population relative to the non-target cell population, and introducing the selected nucleic acid sequences into at least one vector capable of directing expression of the selected nucleic acid sequences in an antigen-presenting cell. In one embodiment, the antigen-presenting cell is a dendritic cell, macrophage, B cell, monocyte or fibrocyte. In another embodiment, the vector further comprises a dendritic cell targeting element.

In one embodiment, the first and second nucleic acid sequences are of the same tissue of origin. The selected nucleic acid sequences can number one or more. For example, the selected nucleic acid sequences can comprise at least 3 different nucleic acid sequences, at least 5 different nucleic acid sequences, at least 7 different nucleic acid sequences, or at least 9 different nucleic acid sequences. The vector can further comprise a nucleic acid sequence encoding an immunomodulatory cofactor. The immunomodulatory cofactor can be, for example, IL-2, IL-3, IL-8, OKT3, α-interferon, γ-interferon, or MIP-1α. The vector can further encode at least one selectable marker. Examples of

selectable markers include, but are not limited to, PLAP, GFP and neomycin resistance. In one embodiment, the target cell is a cancer cell. In another embodiment, the target cell is an infectious agent, such as a virus, a bacterium or a parasite.

The invention additionally provides a composition comprising at least one vector produced by the method described above. In one embodiment, the vector further comprises an antigen-presenting cell targeting element. The composition can further comprise an antigen-presenting cell.

The invention also provides a method of producing an antigen-presenting cell that presents an array of antigens. The method comprises comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population. The method further comprises selecting nucleic acid sequences preferentially expressed by the target cell population relative to the non-target cell population, and genetically modifying an antigen-presenting cell to express the selected nucleic acid sequences. Also provided is an antigen-presenting cell produced by the method described above or genetically modified with a vector produced by a method of the invention.

The invention provides a method of activating immune cells comprising contacting an immune cell with an antigen-presenting cell genetically modified in accordance with the invention. In one embodiment, the immune cell is a T cell. Examples of T cells activated by the method include, but are not limited to, cytotoxic T lymphocytes (CTLs) and helper T cells. Also provided is a method of inducing a toleragenic response comprising contacting an immune cell with an antigen-presenting cell genetically modified in accordance with the invention. In one embodiment, the immune cell is a helper T cell such as a T_{H2} cell. In one embodiment of the method of activating T cells or inducing a toleragenic response, the contacting occurs *in vivo*. Alternatively, the contacting can occur *ex vivo*. The invention also provides immune cells, such as CTLs and T_{H2} cells, activated by a method of the invention. The activated immune cells can be provided in the form of a composition.

The invention provides a method of activating T cells *in vivo* comprising administering a composition comprising a vector or antigen-presenting cell of the invention to a subject. Also provided is a method of killing a target cell *in vivo* comprising administering a composition, vector or antigen-presenting cell of the invention to a subject. The invention also provides methods of preventing infection, treating cancer, and treating a viral infection, the methods comprising administering a composition, vector or antigen-presenting cell of the invention to a subject.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 depicts cell surface markers that can be used to identify dendritic cells. A (-) indicates a marker not present on dendritic cells that can be used in negative selection strategies. The (+), (++) and (+++) respectively indicate increasingly useful markers present on the dendritic cell surface.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a strategy for presentation of multiple antigens in antigen-presenting cells which can be used to generate a prophylactic or therapeutic immune response against one or more target cell populations with which the antigens are associated. The strategy employs comparing and selecting nucleic acid sequences expressed by target and non-target cells. By identifying nucleic acid sequences preferentially expressed in a target cell population and expressing the identified sequences in antigen-presenting cells, one can stimulate an immune response directed at a target cell population without being limited to previously identified antigens. The use of an array of antigens can elicit a more effective immune response by directing lymphocytes to a variety of antigen targets. This multiple antigen strategy is advantageous both for targeting an immune response to more antigens associated with a given disease, and for targeting an immune response to a disease for which an effective antigen for a particular patient or for a particular disease is not known.

The vectors and antigen-presenting cells of the invention can be prepared without prior purification of individual disease-associated antigens. This is particularly useful when a given tissue or cell expresses multiple disease-associated antigens, or when a target antigen is unknown. By making use of differential screening to identify preferentially expressed nucleic acid sequences, one can avoid problems with lack of specificity as nucleic acid sequences encoding non-target antigens can be excluded.

The invention provides a composite preparation of vectors encoding antigens for expression in antigen-presenting cells, which cells can then be used to activate T cells of a subject *in vivo*, *ex vivo*, or *in vitro*, without specific prior knowledge of the relevant disease-associated antigens in that subject. A composite preparation can be used to activate a variety of T cells, directed against more than one antigen and against more than one disease or target cell population.

Definitions

All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

As used herein, "target cell population" means one or more cells sharing at least one common feature, the elimination or protection of which is desired. Examples of a target cell include, but are not limited to, an infectious agent such as a virus, bacterium or parasite, a cell which is susceptible to autoimmune attack, and a disease cell such as a cancer cell, including highly metastatic cancer cells and low metastatic cancer cells. As used in the context of a target cell, "infectious agent" includes both the infectious agent itself and cells infected by the agent. For example, when the target cell is a virus, the target cell may be the virus alone, a virally infected cell or both.

As used herein, "preferentially expressed" refers to nucleic acid sequences that are present in substantially greater amounts in a target sample as compared to a non-target sample. A substantially greater amount can be at least about 20% more. In one

embodiment, substantially greater is about 50% more. In another embodiment, substantially greater is about twice as much. In a preferred embodiment, substantially greater is at least about 10 times as much. In more preferred embodiments, substantially greater is at least about 20, 50 or 100 times as much. The relationship between the target and non-target samples is selected to optimize the identification of sequences encoding antigens relatively specific to the target cells. For example, a target cell can be a colon carcinoma cell and a corresponding non-target cell would be a non-cancerous colon cell. In another example, the target cell can be a highly pathogenic virus and the non-target cell a less pathogenic virus. Differential screening between such target and non-target populations can yield nucleic acid sequences encoding antigens associated with a particular disease or pathogen, without requiring knowledge of the relevant antigens.

As used herein, "vector" means a construct which is capable of delivering, and preferably expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence which directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally-occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence optionally includes the complementary sequence.

As used herein, "antigen-presenting cell" or "APC" means a cell capable of handling and presenting antigen to a lymphocyte. Examples of APCs include, but are not limited to, macrophages, Langerhans-dendritic cells, follicular dendritic cells, B cells, monocytes, fibroblasts and fibrocytes. Dendritic cells are a preferred type of antigen presenting cell. Dendritic cells are found in many non-lymphoid tissues but can migrate via the afferent lymph or the blood stream to the T-dependent areas of lymphoid organs. In non-lymphoid organs, dendritic cells include Langerhans cells and interstitial dendritic cells. In the lymph and blood, they include afferent lymph veiled cells and blood dendritic cells, respectively. In lymphoid organs, they include lymphoid dendritic cells and interdigitating cells. As used herein, each of these cell types and each of their progenitors is referred to as a "dendritic cell," unless otherwise specified.

As used herein, "antigen-presenting cell targeting element" means a molecule which is capable of specifically binding an antigen-presenting cell, such as a dendritic cell. A targeting element specifically binds a dendritic cell when a biological effect is seen in that cell type after binding of the targeting element and its complement, or, when there is greater than a 10 fold difference, and preferably greater than a 25, 50 or 100 fold difference between the binding of the coupled targeting element to dendritic cells and non-target cells. Generally, it is preferable that the targeting element bind to antigen-presenting cells with a K_D of less than 10^{-5} M, preferably less than 10^{-6} M, more preferably less than 10^{-7} M, and most preferably less than 10^{-8} M (as determined by Scatchard analysis (Scatchard, 1949, Ann. N.Y. Acad. Sci. 51:660-672)). Suitable targeting elements are preferably non-immunogenic, not degraded by proteolysis, and not scavenged by the immune system. Particularly preferred targeting elements have a half-life (in the absence of a clearing agent) in an animal of between 10 minutes and 1 week. Examples of dendritic cell surface markers, against which antibodies (or antigen binding domains derived therefrom) can be generated to produce dendritic cell targeting elements, include, but are not limited to, those depicted in Figure 1 (e.g., CD1, CD11a, CD11c, CD23, CD25, CD32, CD40, CD45, CD54, CD58, MHC Class I, MHC Class II, Mac-1, Mac-2, Mac-3).

As used herein, "immunomodulatory cofactor" includes a factor which, when expressed in APCs, causes the immune response to an antigen presented by the APC to be enhanced in quality or potency from that which would have occurred in the absence of the cofactor. The quality or potency of a response may be measured by a variety of assays known in the art including, for example, *in vitro* assays which measure cellular proliferation (e.g., ^{3}H-thymidine uptake), and *in vitro* cytotoxicity assays (e.g., which measure ^{51}Cr release; Warner et al. 1991, AIDS Res. and Human Retroviruses 4:645-655). In alternative embodiments, an immunomodulatory cofactor is, rather than being encoded by the expression vector, added exogenously before, concurrently with, or after administration of the vector. Examples of immunomodulatory cofactors include, but are not limited to cytokines and chemokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, OKT3, α-interferon, β-interferon, γ-interferon, MIP-1α (LD-78), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), tumor necrosis factors (TNFs), CD3, CD8, ICAM-1, ICAM-2, LFA-1, LFA-3, and other proteins such as HLA Class I molecules, HLA Class II molecules, B7, B7-2, β_2-microglobulin, chaperones, and MHC linked transporter proteins or analogs thereof. The choice of immunomodulatory cofactor is based on the therapeutic effects of the factor. Preferred immunomodulatory cofactors include α-interferon, γ-interferon and IL-2.

Nucleic Acid Sequences

The invention provides nucleic acid sequences encoding an array of antigens that are preferentially expressed in a target cell population and which can be used to genetically modify antigen-presenting cells (APCs). The invention further provides a method for preparing these nucleic acid sequences. The nucleic acid sequences can be prepared by comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population. Nucleic acid sequences preferentially expressed by the target cell population relative to the non-target cell population are then selected. This method provides nucleic acid sequences that can be used to express an array of antigens in APCs without prior knowledge of the antigens

and without compromising specificity for the target cell population. Nucleic acid sequences include DNA, RNA, and synthetic derivatives thereof.

Methods of comparing sets of nucleic acid sequences and selecting differentially expressed sequences are known in the art. The comparing can be performed using nucleic acid sequences isolated from samples, such as from one or more cell types, or it can be performed by comparing sequence data obtained, for example, from a public database such as GenBank, EMBL, or DNA Database of Japan (DDBJ). For example, when two nucleic acid samples are to be compared, such as sequences from a target cell population and sequences from a non-target cell population, each sample can be derived from either an isolated pool of nucleic acid sequences, database sequence information, any other source of nucleic acid sequence information, or a combination of two or more sources. Conventional techniques for differential display of mRNA and differential screening of a cDNA library are described in F. M. Ausubel et al., eds., 1996, Current Protocols in Molecular Biology, John Wiley & Sons, Inc., unit 5.8 and sections 5.8.6, 5.8.14.

One example of detecting differential expression of genes is described in U.S. Patent No. 5,677,125, which relates to a method of detecting and diagnosing pre-invasive breast cancer. In this example, epithelial cells are isolated from a sample of abnormal breast tissue which exhibits histological or cytological characteristics of pre-invasive breast cancer, and mRNA is isolated from the sample. One or more abnormal breast tissue cDNA libraries is prepared from the isolated mRNA. One or more cDNA libraries is then prepared by similar means from a sample of normal breast tissue. The cDNA of the abnormal and normal libraries is compared to detect the expression of at least one gene in the abnormal sample which is different from that expressed in the normal sample. A similar approach can be applied to obtain an array of nucleic acid sequences preferentially expressed by a target cell population relative to a non-target cell population for introduction into APCs.

The non-target cell can be a normal cell or it can be a diseased cell, but having a different spectrum of antigens from the target cell. For example, it may be desirable to obtain antigens specific for a particular stage in disease. Thus, the non-target cell could be a cell in a different stage of the target disease. An example of a non-target cell population is one or more selected from stage 1 and stage 2 cells in cervical carcinoma. Antigen-presenting cells can be provided that present antigens specific to a desired stage, such as stage 3 cervical carcinoma. In another example, the target cell population is a highly metastatic colon cancer cell line and the non-target cell population is a low metastatic colon cancer cell line. Differential screening of nucleic acid sequences expressed by the two cell lines can be used to select sequences encoding antigens specific to highly metastatic colon cancer cells. When the non-target cell is a normal cell, differential screening eliminates or reduces the nucleic acid sequences common to normal cells, thereby avoiding an immune response directed at antigens present on normal cells. When the non-target cell is a normal cell, differential screening eliminates or reduces nucleic acid sequences common to normal cells, thereby avoiding an immune response directed at antigens present on normal cells. Examples of target/non-target cell combinations suitable for differential screening include, but are not limited to, KM12L4A cells (high metastatic colon cancer line: Morikawa et al., 1988, Cancer Res. 48:1943)/KM12C (low metastatic colon cancer line; Morikawa et al., 1988, Cancer Res. 48:6863); MDA-MB-231 cells (high metastatic breast cancer line; Brinkley et al., 1980, Cancer Res. 40:3118)/MCF-7 cells (non-metastatic breast cancer line: Yang et al., 1998, Anticancer Res. 18(1A):53-59); and MV-522 cells (high metastatic lung cancer line; Varki et al., 1987, Int. J. Cancer 40:46)/UCP-3 cells (low metastatic lung cancer cell line; Varki et al., 1987, *supra*).

Differential screening can also provide various tailored combinations of antigen-presenting cells. One combination of APCs of the present invention encompasses antigens relating to associated pathologies. For example, HIV-infected patients are often infected with CMV. Accordingly, antigen-presenting cells can be prepared expressing genes preferentially associated with HIV-infected cells, and a second

preparation can be prepared in which the antigen-presenting cells express antigen preferentially expressed by CMV-infected cells. These two preparations can be combined and administered to a subject providing simultaneous treatment for both pathologies. Examples of other associated pathologies wherein preparations of antigen-presenting cells can be combined include HIV and Epstein-Barr virus (EBV) positive lymphoma; and HIV and hepatitis C virus (HCV).

Another example in which separate preparations can be combined provides APCs for targeting a tumor or tumors of unclear origin. In this case, antigen-presenting cells genetically modified to express nucleic acid sequences from a spectrum of tumor types can be used to elicit a broad anti-tumor immune response.

A third instance in which separate preparations can be combined provides for targeting a disease associated with a different spectrum of antigens in different patients. Preparations from various patients that encompass most or all of the repertoire of antigens associated with the disease in most or all patients can be combined and administered to a subject without having to first determine what antigens are expressed in that individual subject's disease.

The combinations can be prepared at the nucleic acid level. In this case, preferentially expressed nucleic acids are combined and then introduced into antigen-presenting cells *in vitro*. Alternatively, preferentially expressed nucleic acids can be used to prepare separate antigen-presenting cells which then can be combined to provide a composite antigen preparation.

In one embodiment of the invention, mRNA preferentially expressed by the target cell population is used to obtain nucleic acid such as cDNA encoding full-length sequences. Nucleic acid sequences so obtained can be used to identify differentially expressed antigens associated with the target cell population. One can then test antigens so obtained to determine which are most effective as immunogens.

In one example of testing an antigen's immunogenicity, peripheral blood cells are removed from a subject. Dendritic cells are then isolated and either pulsed with the antigen to be tested or genetically modified to express the test antigen. The pulsed or modified dendritic cells can then be used to stimulate the subject's T cells *in vitro*. The ability of the modified dendritic cells to stimulate T cells is indicative of immunogenicity of the test antigen.

Vectors

The invention additionally provides vectors containing nucleic acid sequences selected by differential screening as described above. The selected sequences can be introduced into at least one vector. Preferably the vector is capable of directing expression of the selected nucleic acid sequences in an antigen-presenting cell, such as a dendritic cell. A vector can encode a single antigen or multiple antigens. When multiple antigens are encoded by a single expression vector, the antigens can be derived from the same or different cell types. Alternatively, a composition comprising more than one vector, each encoding one or more antigens associated with a cell type the same as or different from those encoded by other vectors, can also be prepared.

In one embodiment, the invention provides a method of producing at least one vector encoding an array of antigens for expression in an antigen-presenting cell. In one embodiment, the method comprises comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population. The method further comprises selecting nucleic acid sequences preferentially expressed by the target cell population relative to the non-target cell population, and introducing the selected nucleic acid sequences into at least one vector capable of directing expression of the selected nucleic acid sequences in an antigen-presenting cell. In one embodiment, the antigen-presenting cell is a dendritic cell, macrophage, B cell, monocyte or fibrocyte. In another embodiment, the vector further comprises a dendritic cell targeting element.

In one embodiment of the method, the first and second nucleic acid sequences are of the same tissue of origin. The selected nucleic acid sequences can number one or more. For example, the selected nucleic acid sequences can comprise at least 3 different nucleic acid sequences, at least 5 different nucleic acid sequences, at least 7 different nucleic acid sequences, or at least 9 different nucleic acid sequences. The vector can further comprise a nucleic acid sequence encoding an immunomodulatory cofactor. The immunomodulatory cofactor can be, for example, IL-2, IL-3, IL-8, OKT3, α-interferon, γ-interferon, or MIP-1α. The vector can further encode at least one selectable marker. Examples of selectable markers include, but are not limited to, PLAP (U.S. Patent Application Serial No. 09/006,298, filed January 13, 1998), GFP and neomycin resistance. In one embodiment, the target cell is a cancer cell. In another embodiment, the target cell is an infectious agent, such as a virus, a bacterium or a parasite.

Vectors of the invention can be used to genetically modify APCs such as dendritic cells either *in vivo* or *in vitro*. Several ways of genetically modifying APCs are known, including transduction with a viral vector either directly or via a retroviral producer cell, calcium phosphate precipitation, fusion of the recipient cells with bacterial protoplasts containing the DNA, treatment of the recipient cells with liposomes containing the DNA, DEAE dextran, receptor-mediated endocytosis, electroporation, micro-injection, and many other techniques known to those of skill. See, e.g., Methods in Enzymology, 185, Academic Press, Inc., San Diego, CA (D.V. Goeddel, ed.) 1990, or M. Krieger, Gene Transfer and Expression -- A Laboratory Manual, Stockton Press, New York, NY, 1990, and the references cited therein, as well as Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook et al. Molecular Cloning - A Laboratory Manual (2nd ed.) 1-3 1989; and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement), WO 93/24640; Mannino and Gould-Fogerite 1988, Biotechniques 6(7):682-691; U.S. Patent No. 5,279,833; WO 91/06309; and Felgner et al. Proc. Natl. Acad. Sci. USA 84:7413-7414 1987.

Examples of viral vectors include, but are not limited to retroviral vectors based on, e.g., HSV, HIV, murine retroviruses, gibbon ape leukemia virus and other viruses such as adeno-associated viruses (AAVs) and adenoviruses. (Miller et al. 1990, Mol Cell Biol. 10:4239; J. Kolberg 1992, NIH Res. 4:43, and Cornetta et al. 1991, Hum. Gene Ther. 2:215). Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), ecotropic retroviruses, human immunodeficiency virus (HIV), and combinations. See, e.g. Buchscher et al. 1992, J. Virol. 66(5):2731-2739; Johann et al. 1992, J. Virol. 66(5):1635-1640; Sommerfelt et al. 1990, Virol. 176:58-59; Wilson et al. 1989, J. Virol. 63:2374-2378; Miller et al. 1991, J. Virol. 65:2220-2224, and Rosenberg and Fauci 1993 in Fundamental Immunology, Third Edition, W.E. Paul (ed) Raven Press, Ltd., New York and the references therein; Miller et al. 1990, Mol. Cell. Biol. 10:4239; R. Kolberg 1992, J. NIH Res. 4:43; and Cornetta et al. 1991, Hum. Gene Ther. 2:215.

Murine vectors comprising Gibbon Ape Leukemia Virus (GaLV) envelopes can be used to transduce many mammalian cells. See, Johann et al. 1992, *supra*. The same receptor is used by simian sarcoma associated virus (SSAV), a strain of GaLV (Sommerfelt et al. 1990, *supra*). The construction of hybrid virions having GaLV envelope proteins has been demonstrated (Wilson et al. 1989, *supra*; Miller et al. 1991, *supra*). Any of these vectors and methods of making retroviral clones can be applied to the present invention. GaLV retroviral packaging cell lines can be used to provide infectious replication-defective hybrid virions for use in gene transfer in humans, hamsters, cows, cats, dogs, monkeys, chimpanzees, macaques, primates, and other species whose cells have host cell receptors for GaLV envelope proteins.

Additional examples of vectors include, but are not limited to adenoviral vectors, AAV vectors, pox viral vectors (B. Moss, 1992, Current Topics in Microbiology and Immunology 158:25-38) including vaccinia, fowl pox, and canary pox, recombinant influenza viral vectors (A. Garcia-Sastre and P. Palese 1995, Biologicals 23:171-178) or non-viral gene delivery techniques (F. Leedley 1994, Biotechnology 5:626-636). AAV-based vectors can be used to transduce cells with selected nucleic acids, e.g., in the *in*

vitro production of nucleic acids and peptides, and in *in vivo* and *ex vivo* gene therapy procedures. See, West et al. 1987, Virology 160:38-47; U.S. Patent No. 4,797,368; WO 93/24641; Kotin 1994, Human Gene Therapy 5:793-801; and Muzyczka 1994, J. Clin. Invest. 94:1351.

In vitro amplification techniques suitable for amplifying sequences to be subcloned into an expression vector are known. Examples of such *in vitro* amplification methods, including the polymerase chain reaction (PCR), ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), are found in Berger, Sambrook et al. 1989, Molecular Cloning - A Laboratory Manual (2nd Ed) 1-3; and U.S. Patent No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA 1990; Arnheim & Levinson (October 1, 1990) C&EN 36-47; J. NIH Res. 1991, 3:81-94; Kwoh et al. 1989, Proc. Natl. Acad. Sci. USA 86:1173; Guatelli et al. 1990. Proc. Natl. Acad. Sci. USA 87:1874; Lomell et al. 1989, J. Clin. Chem. 35:1826; Landegren et al. 1988, Science 241:1077-1080; Van Brunt 1990, Biotechnology 8:291-294; Wu and Wallace 1989, Gene 4:560; Barriner et al. 1990, Gene 89:117; and Sooknanan and Malek 1995, Biotechnology 13:563-564. Improved methods of cloning *in vitro* amplified nucleic acids are described in U.S. Patent No. 5,426,039.

SUBSTITUTE SHEET (RULE 26)

Antigen-Presenting Cells

Antigen-presenting cells (APCs) process antigen and present it to a lymphocyte. The invention provides APCs that present an array of antigens. The APCs are genetically modified to express nucleic acid sequences preferentially expressed by a target cell population relative to a non-target cell population. Examples of APCs include, but are not limited to, macrophages, Langerhans dendritic cells, follicular dendritic cells, B cells, monocytes, fibroblasts and fibrocytes. In a preferred embodiment, the APC is a dendritic cell. Selection of the optimal APC can vary, however, with the antigen to be presented (Butz and Bevan 1998, J. Immunol. 160:2130-2144).

Dendritic cells have an unusual dendritic shape, are motile, and efficiently cluster and activate T cells that are specific for cell surface stimuli. Typically, dendritic cells in non-lymphoid organs, such as Langerhans cells and interstitial cells, become veiled cells (cells which continually extend and retract large lamellipodia) in the afferent lymph and blood which migrate to lymphoid tissues, where they can be isolated as dendritic or interdigitating cells.

Dendritic cells initiate T-dependent responses from quiescent lymphocytes. Once sensitized, T cells interact with other antigen presenting cells. Dendritic cell antigen processing activity is regulated. Fresh cells, i.e., cells cultured for less than a day, isolated from skin or lymphoid organs present native proteins. After that time, they do not process antigens. In addition, dendritic cells are not actively phagocytic.

Dendritic cells can be isolated and prepared using conventional techniques such as those described in Tedder and Jansen, 1997, Current Protocols in Immunology, John Wiley & Sons, unit 7.32. Other methods for obtaining and identifying dendritic cells are described in WO 98/15579; WO 98/01538; WO 98/15615; and WO 98/14561. For example, human dendritic cells can be isolated from blood mononuclear cells by first enriching a peripheral cell population for dendritic cells by depletion of T cells and adherent cells. Density gradient centrifugation of the preparation over metrizamide is used to isolate low buoyant density cells. This population has virtually no lymphocytes and contains 20-80% dendritic cells. The purity of dendritic cells can be determined by a variety of techniques, including hemacytometer counting, immunostaining after cytocentrifugation onto glass slides and immunofluorescence staining with flow cytometric analysis. Flow cytometry is preferred, however, for optimal quantitation and consistency.

Young et al. have identified dendritic cell colony-forming units among normal human CD34+ (positive for this hematopoietic stem cell marker) bone marrow progenitors that give rise to pure dendritic cell colonies (Young et al. 1995, J. Exp. Med. 182:1111-1120). Addition of c-*kit*-ligand to GM-CSF- and TNF-α-supplemented suspension of

CD34+ bone marrow cells expands dendritic cell colony-forming units almost 100-fold by 14 days. These colony-derived dendritic cells are potent stimulators of T cells.

Partially enriched populations of epidermal Langerhans cells, wherein Langerhans cells may comprise up to about 60% of the total cell population, may be readily prepared. Keratinocytes can be depleted from murine tissue using α-thy-1 (a monoclonal antibody) and complement plus adherence. Enriched preparations of human Langerhans cells can be prepared by substituting an anti-CD 1 antibody for α-thy-1. In culture, neither mouse nor human Langerhans cells are active antigen-presenting cells until after 1-3 days in culture, after which time they enlarge, express more MHC Class II and cell adhesion molecules, and lose Fc receptors, fully resembling blood and lymphoid dendritic cells. Cell populations containing more than 90% dendritic cells have been obtained from human blood, where, without enrichment, fewer than 0.1% of the white cells are dendritic cells. Such enrichment can be achieved by successive depletion of T cells, monocytes, and B plus NK cells to yield an initial population ranging from 30-60% dendritic cells. Greater purity is then obtained by panning or fluorescence activated cell sorting (FACS) using a monoclonal antibody, especially to $CD45R_A$, that selectively reacts to contaminants (P. Freudenthal, et al. 1990, Proc. Natl. Acad. Sci. (USA) 87:7698-7702). To enrich for dendritic cells generally, selection for low buoyant density, non-adherence to plastic in culture (especially after one or more days), and absence of markers found on other cells is performed. Such methods deplete other cell types, but do not positively select dendritic cells.

Dendritic cells express a distinct pattern of markers on their cell membranes. Figure 1 illustrates this pattern by indicating the presence or absence of several distinct cell surface markers. Other markers which can be used to positively or negatively select for dendritic cells include ICAM-1 (CD54), LFA-3 (CD58), and CD 11b. Dendritic cells isolated from human or mouse blood, but not skin, express CD 11a or LFA-1. In skin, the immunostimulatory effect of dendritic cells may be enhanced by cytokines, particularly by GM-CSF.

Dendritic cells, or other APCs, can be selected to obtain a population comprised substantially of dendritic cells, i.e., greater than about 50% dendritic cells, more preferably greater than about 75% dendritic cells, more preferably still greater than about 90% dendritic cells, with greater than about 95% dendritic cells being particularly preferred. The antigen-presenting cells are preferably isolated from the subject into which the activated T cells are to be active ("autologous" therapy). Alternatively, the cells can be obtained from a donor or a cell bank (e.g., a blood bank).

The invention provides a method for preparing antigen-presenting cells that present an array of antigens. APCs can be genetically modified using one or more vectors of the invention. Antigen-presenting cells are transduced with vectors encoding an array of antigens. These antigens are then expressed in the cells, processed by the cells, and the relevant processed antigen fragment is routed to the cell surface where it can be presented.

The culture of cells used in conjunction with the present invention, including cell lines and cultured cells from tissue or blood samples, including dendritic cells is well known in the art. Freshney (Culture of Animal Cells, a Manual of Basic Technique, third edition WIley-Liss, New York (1994)) and the references cited therein provides a general guide to the culture of cells.

T Cells

T cells can be isolated and activated *in vitro* by contact with an antigen-presenting cell. Several such techniques are well-known. The expression of surface markers facilitates identification and purification of T cells. Methods of identification and isolation of T cells include FACS, incubation in flasks with fixed antibodies which bind the particular cell type and panning with magnetic beads.

T cells and dendritic cells are characterized by expression of particular markers on the surface of the cell, and lack of expression of other markers. For instance, dendritic cells express MHC molecules and costimulatory molecules (e.g., B7-1 and B7-2), a lack of

markers specific for granulocytes, NK cells, B cells, and T cells. In the mouse, some, but not all, dendritic cells express 33D1 (dendritic cells from spleen and Peyer's patch, but not skin or thymic medulla), NLDC 145 (dendritic cells in skin and T-dependent regions of several lymphoid organs and CD11c) (CD11c also reacts with macrophage). T cells are positive for various markers depending on the particular subtype, most notably CD4 and CD8.

Cell isolation or immunoassays for detection of cells during cell purification can be performed in any of several configurations, e.g., reviewed in Maggio (ed.) 1980, Enzyme Immunoassay CRC Press, Boca Raton, Florida; Tijan 1985, "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam; Harlow and Lane, *supra*; Chan (ed.) 1987, Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) 1991, Principles and Practice of Immunoassays Stockton Press, NY; and Ngo (ed.) 1988, Non-isotopic Immunoassays Plenum Press, NY. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) 1991, Basic and Clinical Immunology (7th ed.). For a discussion of how to make antibodies to selected antigens, see, e.g., Coligan 1991, Current Protocols in Immunology Wiley/Greene, N.Y.; and Harlow and Lane 1989, Antibodies: A Laboratory Manual Cold Spring Harbor Press, N.Y.; Stites et al. (eds.) Basic and Clinical Immunology (4th ed.).

Most preferably, cells are isolated and characterized by flow cytometry methods using fluorescence activated flow cytometry (FACS). A wide variety of flow-cytometry methods are known. For a general overview of FACS see, for example, Abbas et al. 1991, Cellular and Molecular Immunology, W.B. Saunders Company, particularly chapter 3, and Kuby 1992, Immunology, W.H. Freeman and Company, particularly chapter 6.

Methods

The invention provides methods for using antigen-presenting cells that present an array of antigens. In one embodiment, the invention provides a method of activating immune cells *in vivo*. Examples of immune cells include, but are not limited to, T cells, including cytotoxic T lymphocytes and helper T cells. In one embodiment, the method comprises administering to a subject a vector encoding an array of antigens preferentially expressed by a target cell population as compared with a non-target population. The vector is preferably constructed so as to be capable of directing expression of the preferentially expressed antigens in an antigen-presenting cell. Preferably, the antigen-presenting cell is a dendritic cell. The vector can be further modified, as described above, to encode an immunomodulatory cofactor. The target cell can be, for example, a cancer cell, virus, bacterium, parasite, or other disease-associated cell. Upon administration, the vector transduces an APC in the subject, thereby genetically modifying an APC *in vivo*. The genetically modified APC is then available to contact and activate an immune cell.

In another embodiment, the method of activating immune cells comprises contacting an immune cell with an antigen-presenting cell genetically modified to express an array of antigens preferentially expressed by a target cell population as compared with a non-target population. Preferably, the antigen-presenting cell is a dendritic cell. The contacting can occur *in vivo* or *ex vivo*. Examples of eliciting an *in vitro* CTL response are provided by S. Nair et al., 1993, J. Virol. 68:5685 and F.J. Rouse et al., 1994, J. Virol. 68:5685. Example of stimulating an *in vivo* T cell response are provided by A. Porgador et al., 1996, J. Immunol. 156(8):2918-2926; E.C. McKinney and J.W. Streilein, 1989, J. Immunol. 143:1560; and H. Takahashi et al., 1993, Int. Immunol. 5:849. For *in vivo* contact, the APC is administered to a subject. Alternatively, immune cells are isolated from a subject and brought into contact with the APC *in vitro* or *ex vivo*. The immune cells, such as T cells, are activated *ex vivo* and then can be reintroduced into the subject or provided to a different subject where they can then come in contact with target cells in that subject. Techniques for adoptive

immunotherapy of cancer are described and reviewed in Chang, A.E. and S. Shu, 1996, Crit. Rev. Oncol. Hematol. 22(3):213-228; and Kradin, R.K., 1993, in Therapeutic Applications of Interleukin-2, Atkins, M.B. and J.W. Mier, eds., Marcel Dekker, Inc. NY, pp. 217-232.

The activation of immune cells by the above methods can, in some embodiments, be used to kill target cells. Thus, the invention provides a method for killing a target cell comprising contacting an immune cell with an APC genetically modified in accordance with the invention. The contacting can be effected *in vivo* by administering a genetically modified APC or by administering a vector of the invention which transduces an APC within the subject, which then contacts an immune cell. Alternatively, the contacting can occur *in vitro*. Immune cells activated *in vitro* by contact with genetically modified APCs can be administered to a subject.

Alternatively, the selection of vectors or APCs can be designed to obtain a toleragenic response, for example, by contacting the APC with a T_{H2} cell. Thus, the invention additionally provides a method of inducing a toleragenic response comprising contacting an immune cell with an APC genetically modified in accordance with the invention. Inducing a toleragenic response can be useful for such applications as treatment of autoimmune disorders and inhibiting rejection of foreign tissue, such as transplant tissue or autologous cells which have been genetically modified with foreign material. As with the methods for killing a target cell, the methods for inducing a toleragenic response can be effected by APCs genetically modified *in vitro* or *in vivo*.

The invention additionally provides a method of preventing disease such as infection or cancer comprising administering to a subject a composition comprising a vector or APC of the invention. Also provided is a method of treating disease such as cancer or infection comprising administering to a subject a composition comprising a vector or APC of the invention. Examples of infections include, but are not limited to, viral, bacterial and parasitic infections. Examples of cancers include, but are not limited to, melanoma, glioma, and cancers of the colon, breast, prostate, lung and liver.

Compositions

The invention provides compositions which are useful for treating and preventing disease, such as cancer or infection. In one embodiment, the composition is a pharmaceutical composition. The composition can comprise a therapeutically or prophylactically effective amount of a vector and/or antigen-presenting cell of the invention, as described above. The composition can optionally include a carrier, such as a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention. Most typically, quality controls (microbiology, clonogenic assays, viability tests), are performed and the cells are reinfused back to the patient, preceded by the administration of diphenhydramine and hydrocortisone. See, for example M. Korbling, et al. 1986, Blood 67:529-532 and Haas et al. 1990. Exp. Hematol. 18:94-98.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, and carriers include aqueous isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Intravenous or intraperitoneal administration is a preferred method of administration.

Methods Of Administration

In one embodiment of the invention, a patient infected with a virus such as HIV-1 or suffering from a cancer such as a melanoma can be treated by administering genetically modified antigen-presenting cells, or by using genetically modified antigen-presenting cells to activate a population of T cells against the infection or cancer, and introducing the T cells back into the patient. Thus, the present invention provides a method of

producing cytotoxic T cells *in vitro*, *ex vivo* or *in vivo*. In another embodiment, the patient is treated by administering at least one vector encoding an array of antigens, wherein the vector includes a dendritic cell target element. The patient's own dendritic cells can then be genetically modified *in vivo*.

T cells such as CD8+ CTLs activated *in vitro* can be introduced into a subject where they are cytotoxic against target cells bearing antigenic peptides corresponding to those the T cells are activated to recognize on class I MHC molecules. These target cells are typically cancer cells, or infected cells which express unique antigenic peptides on their MHC class I surfaces.

Similarly, helper T cells (e.g., CD4+ T cells), which recognize antigenic peptides in the context of MHC class II, are also stimulated by genetically modified antigen-presenting cells (e.g., dendritic cells), which can comprise antigenic peptides both in the context of class I and class II MHC. These helper T cells also stimulate an immune response against a target cell. As with cytotoxic T cells, helper T cells are stimulated with the genetically modified antigen-presenting cells *in vitro* or *in vivo*. In one embodiment, a toleragenic response is generated by administration of genetically modified APCs via the stimulation of T_{H2} cells.

Arrays of antigens are preferably associated with diseases selected from the group consisting of cancer, a hyperproliferative disease, a bacterial infection, a parasitic infection, and a viral infection. Diseases suitable for treatment using an immunostimulation strategy include: viral infections, such as those caused by HBV (see WO 93/15207), HCV (see WO 93/15207), HPV (see WO 92/05248, WO 90/10459, EPO 133,123), Epstein-Barr Virus (see EPO 173,254; JP 1,128,788; and U.S. Patent Nos. 4,939,088 and 5,172,414), Feline Leukemia Virus (see WO 93/09070, EPO 377,842, WO 90/08832, and WO 93/09238), Feline Immunodeficiency Virus (U.S. Patent No. 5,037,753, WO 92/15684, WO 90/13573, and JP 4,126,085), HTLV I and II, and HIV (see WO 91/02805); cancers, such as melanoma, cervical carcinoma, colon

carcinoma, renal carcinoma, breast cancer, ovarian cancer, prostate cancer, leukemias; and heart disease.

Bacterial infections that may be treated include, but are not limited to, pneumonia, sepsis, tuberculosis, and *Staphylococcus* infections, among others.

Parasitic infections that can be treated include, but are not limited to, malaria (caused by protozoa of the genus *Plasmodium*, and include *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*), sleeping sickness (caused by trypanosomes), and river blindness.

Viral infections that can be treated include, but are not limited to, those caused by hepatitis A, hepatitis B, hepatitis C, non-A, non-B hepatitis, hepatitis delta agent, CMV, Epstein-Barr virus, HTLV 1, HTLV II, FeLV, FIV, and HIV I.

Treatment includes prophylaxis and therapy. Prophylaxis or treatment can be accomplished by a single direct injection at a single time point or multiple time points. Administration can also be nearly simultaneous to multiple sites.

Patients or subjects include mammals, such as human, bovine, equine, canine, feline, porcine, and ovine animals.

In one embodiment, T cells or antigen-presenting cells are administered directly to the subject to produce T cells active against a target cancerous, infected, or other cell type. Administration of these is by any of the routes normally used for introducing a cell into ultimate contact with a mammal's blood or tissue cells. In another embodiment, at least one vector encoding an array of antigens is administered.

Compositions are typically administered *in vivo* via parenteral (e.g. intravenous, subcutaneous, and intramuscular) or other traditional direct routes, such as buccal/sublingual, rectal, oral, nasal, topical, (such as transdermal and ophthalmic), vaginal, pulmonary, intraarterial, intraperitoneal, intraocular, or intranasal routes or directly into a specific tissue, such as the liver, bone marrow, or into the tumor in the case of cancer therapy. Non-parenteral routes are discussed further in WO 96/20732.

The cells or vectors are administered in any suitable manner, often with pharmaceutically acceptable carriers. Suitable methods of administering cells in the context of the present invention to a patient are available, and, although more than one route can be used to administer a particular cell composition, a particular route can often provide a more immediate and more effective reaction than another route.

The dose of cells (e.g., activated T cells, or dendritic cells) administrated to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time, or to inhibit growth of cancer cells, or to inhibit infection. Thus, cells are administered to a patient in an amount sufficient to elicit an effective immune response to the specific antigens and/or to alleviate, reduce, cure or at least partially arrest symptoms and/or complications from the disease or infection. An amount adequate to accomplish this is defined as a "therapeutically effective dose."

The dose will be determined by the activity of the T cell or antigen-presenting cell produced and the condition of the patient, as well as the body weight or surface areas of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of a particular cell in a particular patient. In determining the effective amount of the cell to be administered in the treatment or prophylaxis of diseases such as AIDS or cancer (e.g., metastatic melanoma, prostate cancer, etc.), the physician needs to evaluate circulating plasma levels, CTL toxicity, progression of the disease, and the production of immune response against any introduced cell type.

Generally at least about 10^4 to 10^6 and typically, between 1 x 10^8 and 1 x 10^{10} cells are infused intravenously or intraperitoneally into a 70 kg patient over roughly 60-120 minutes. Intravenous infusion is preferred. Vital signs and oxygen saturation by pulse oximetry are closely monitored. Blood samples are obtained 5 minutes and 1 hour following infusion and saved for analysis. Cell reinfusions are repeated roughly every month for a total of 10-12 treatments in a one year period. After the first treatment, infusions can be performed on an outpatient basis at the discretion of the clinician. If

the reinfusion is given as an outpatient, the participant is monitored for at least 4 hours following the therapy.

For administration, cells of the present invention can be administered at a rate determined by the LD-50 (or other measure of toxicity) of the cell type, and the side-effects of the cell type at various concentrations, as applied to the mass and overall health of the patient. Administration can be accomplished via single or divided doses. The cells of this invention can supplement other treatments for a condition by known conventional therapy, including cytotoxic agents, nucleotide analogues and biologic response modifiers. Similarly, biological response modifiers are optionally added for treatment. For example, the cells are optionally administered with an adjuvant, or cytokine such as GM-CSF, IL-12 or IL-2.

Administration by many of the routes of administration described herein or otherwise known in the art may be accomplished simply by direct administration using a needle, catheter or related device, at a single time point or at multiple time points. In addition, an "administration" of a gene delivery vehicle (or *ex vivo* transduced cells, for that matter) at a given time point includes administration to one or more areas, or by one or more routes. In certain embodiments of the invention, one or more dosages is administered directly in the indicated manner: intravenously at dosage greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu; intraarterially at dosages greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu; intrasmuscularly at dosages greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu, with dosages of 10^{10} or 10^{11} cfu being preferred; intradermally at dosages greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu; pulmonarily at dosages greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu; subcutaneously at dosages greater than or equal to 10^3, 10^5, 10^7, 10^9, 10^{10} or 10^{11} cfu, with dosages of 10^9, 10^{10} or 10^{11} cfu being preferred; interstitially at dosages greater than or equal to 10^3, 10^5, 10^7, 10^8, 10^9, 10^{10} or 10^{11} cfu, with dosages of 10^8, 10^9, 10^{10} or 10^{11} cfu being preferred; into a lymphoid organ such as the spleen, a tonsil, or a lymph node at dosages greater than or equal to 10^3, 10^5, 10^7, 10^8, 10^9, 10^{10} or 10^{11} cfu; into a tumor at dosages greater than or equal to 10^3, 10^4, 10^5, 10^5, 10^7, 10^8, 10^9, 10^{10} or 10^{11} cfu,

with dosages of 10^8, 10^9, 10^{10} or 10^{11} cfu being preferred; and into the afferent lymph at dosages greater than or equal to 10^3, 10^5, 10^7, 10^8, 10^9, 10^{10} or 10^{11} cfu. For purposes of the convenience, "cfu" shall also refer to non-viral particles, such that one cfu is equivalent to one-non-viral particle.

EXAMPLES

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1: Preparation of dendritic cells genetically modified to present an array of antigens

Dendritic cells are isolated according to Basic Protocol I described in Tedder and Jansen, 1997, Current Protocols in Immunology, John Wiley and Sons, 7.32.1. Cell lines from a matched pair of target and non-target cell types are obtained. A matched pair of cell types includes, for example, cell lines derived from the same tissue of origin, such as prostate, and differing in a targeted feature. An example of a target cell is a prostate cancer cell. An example of a matched pair of cell types is prostate cells differing in their metastatic potential. Nucleic acid sequences preferentially expressed in prostate cancer tissue are described in United States patent application serial number 60/088,877, filed June 11, 1998, the entire contents of which are incorporated herein by reference. The dendritic cells are then transduced with the preferentially expressed nucleic acid sequences using conventional techniques, such as those described in WO 97/24447, the entire contents of which are incorporated herein by reference. Successful transduction of the human dendritic cells can be confirmed by in vitro T cell priming (Albert et al., 1998, Nature 392:86-89; Nair et al., 1998, Nature Biotechnology 16(4):364-369; Antigen Processing and Presentation, in Current Protocols in Immunology, Wiley, New York, 1998). Candidate immunogenic tumor-associated antigen sequence arrays can be determined by screening transduced dendritic cells with responding T cells obtained from peripheral blood or dLN of tumor-bearing patients.

Once a pattern of reactivity is found in a particular tumor type, the relative efficacy of these antigens can be evaluated using murine homologs and syngeneic murine tumor models that express one or more of these antigens. Example 2 describes one method for evaluating efficacy.

Example 2: Dendritic cell-based immunotherapy for the treatment of metastatic tumors in combination with systemic Proleukin® IL-2

Genetically modified dendritic cells of the invention can be used to treat tumors, alone or in combination with other therapies. This example shows how to evaluate the contribution of dendritic cells genetically modified using murine homologs of nucleic acid sequences identified by the methods of Example 1 to systemic Proleukin IL-2 immunotherapy on murine syngeneic tumors. The C57B/6 derived B16-F10 lung metastasis model, CT-26 murine colon model or the murine 3LL lung model can be used to generate the most effective treatment regimen. These tumor models are poorly immunogenic and are differentially responsive to single agent Proleukin therapy, as measured by both survival and tumor load.

The research strategy can be used to define the optimal mixture of dendritic cells and systemic Proleukin IL-2, e.g. a lower amount of IL-2 to maintain a high objective response rate while lowering toxicities, and to develop a dendritic cell plus systemic IL-2 regimen for application to a lung and/or colon murine syngeneic tumor model that is resistant to single agent IL-2 therapy.

Experimental Design:

Murine splenic dendritic cells are isolated and characterized as described by Girolomoni et al. 1990, J. Immunol. 145(9):2820-26. First, spleens from either naïve or day 7 tumor bearing mice are removed, minced, and digested in Hank's Balanced Salt Solution (HBSS) with 40 mg collagenase (Sigma, St. Louis, MO) for 1 hour. Cells are then filtered over 100 μm nylon mesh; and washed. Red blood cells are lysed with 0.83% Ammonium chloride, 0.1% $KHCO_2$, 0.004% EDTA (ACK Lysis buffer); pellet

resuspended to 3 x 10^7 cells/ml in 1.035 Percoll (Pharmacia biotech) and underlay with an equal volume of 1.075 g/ml Percoll. The suspension is centrifuged at 2200 rpm for 20 minutes at 4°C. The band is harvested at interface, washed twice with HBSS, and resuspended to 5 x 10^6 cells/ml in complete culture medium and incubated at 37°C for 90 min. Nonadherent cells are removed and discarded. Fresh complete culture medium is added, and culture continued for 18-24 hr at 37°C. Gentle pipetting dislodges splenic dendritic cells.

Cells harvested from the spleen cell cultures are further enriched by overlaying 2 mls of 5 x 10^6/ml cells onto a 3 ml layer of 14.5% Metrizamide-CM solution in a 15 ml centrifuge tube. The gradient is centrifuged at 2000 rpm for 15 min at 4°C, the band harvested, and washed, counted, and resuspended to 5 x 10^6 cells/ml in complete medium.

An aliquot can be removed for phenotyping using the following 3-color staining protocol ("DC" refers to dendritic cells):

Tube 1	CD3/Isotype	Isotype control
Tube 2	CD3/CD4/CD8	CD4/CD8 T cells
Tube 3	CD45/I-A^b/CD86	CD vs monocytes
Tube 4	CD45/CD80/CD40	DC vs monocytes
Tube 5	CD45/CD11b (Mac-1)/CD11c	DC vs monocytes
Tube 6	CD45/CD45R(B22))/CD44	DC vs B cells

Bone marrow derived dendritic cells (BM-DC) are isolated from erythrocyte depleted mouse bone marrow cells cultured for seven days in complete medium supplemented with 10 ng/ml GM-CSF and 10 ng/ml IL-4. On day seven, BM-DC are harvested by gentle pipetting and further enriched by 14.5% (by weight) metrizamide (Sigma, St. Louis, MO) complete medium gradients. The low density interface containing the BM-DC are collected by gentle pipette aspiration. The BM-DC are washed twice with complete medium, enumerated (purity >90%, positive co-expression of MHC Class II, CD40, CD80, CD86 and CD11c).

Genetically modified dendritic cells prepared as described in Example 1 and/or 2 are then injected either before (protective) or after (therapeutic) tumor challenge. For protection, an intravenous (iv) tumor challenge is introduced 1 week following the last immunization (days -15, -8, 0). For therapy, immunizations are given at days 4, 8, and 12 post-iv tumor challenge, with or without Proleukin.

Proleukin therapy is administered as follows:

For protection: 10 mg/kg iv for 5 days starting with second immunization.

For therapy: 10 mg/kg/day iv for 5 or 10 days starting on day 2 post-iv tumor challenge.

Experiment #1: Single agent Proleukin therapy (10 mice/group):

B16-F10: 50-85,000 cells iv D0

Proleukin: iv twice per day for seven days, beginning at Day 3

Sacrifice and count lung metastases

Group	1	B16	PBS control
	2	B16	2.5 mg/kg Proleukin
	3	B16	3.5 mg/kg Proleukin
	4	B16	5 mg/kg Proleukin

Experiment #2: Take best Proleukin regimen (<2/10 dead mice with >50% reduction in lung metastases).

Group	1	B16	PBS
	2	B16	Proleukin
	3	B16	modified DC alone
	4	B16	modified DC + Proleukin
	5	B16	DC- alone
	6	B16	DC- + Proleukin
	7	B16	irradiated B16 alone (50,000/iv)

8	B16	irradiated B16 + Proleukin

Experiment #3: Take most active DC regimen (double single agent Proleukin lung metastases reduction)

Group 1	B16	PBS
2	B16	Proleukin
3	B16	modified DC alone
4	B16	modified DC + Proleukin
5	B16	modified DC + 75% Proleukin
6	B16	modified DC + 50% Proleukin
7	B16	modified DC + 25% Proleukin

The potency of the genetically modified dendritic cells (DC) can be evaluated with a short term culture immune function assay, based on the antigen-induced expression of CD69. Briefly, whole blood and/or spleen cells from naïve, tumor-bearing, and DC-immunized mice are prepared as single cell preparations, depleted of macrophages by adherence, and resuspended to 5 x 10^6 cells/ml. Five hundred microliters of various DC sources (x-irradiated) are titrated into 12 x 75 mm polypropylene tubes containing 0.5 ml of the monocyte-depleted responding cells. The mixed cells are cultured for 24 hours at 37°C, with a 6 hr timepoint taken for analysis. The cultures contain Brefeldin for the last 4 hours of culture. The cultures are then surfaced-stained with CD3/CD4/CD69 or CD3/CD8/CD69. Duplicate cultures are permeabilized, and stained with CD3/CD4/IFNγ, CD3/CD4/IL-4, CD3/CD8/IFNγ, and CD3/CD8/IL-4. Reagents for TNFα and GM-CSF are used to further define the CD8 reactivity to the genetically modified DC.

DC loaded with baculovirus-produced mouse gp100 (cloned out from B16F10) are used as a positive control.

Serum from the immunized mice can be obtained, pelleted, heat-inactivated, and stored at -70°C for future analysis of anti-tumor binding activity, cytokine/chemokine content, sIL-2R, and other inflammatory molecules.

The foregoing detailed description provides exemplary information about the invention. Those skilled in the art will appreciate that modifications can be made without diverging from the spirit and purpose of the invention.

Substitute Sheet (Rule 26)

CLAIMS

What is claimed is:

1. A method of producing at least one vector encoding an array of antigens for expression in an antigen-presenting cell comprising:

(a) comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population;

(b) selecting nucleic acid sequences preferentially expressed by the target cell population relative to the non-target cell population; and

(c) introducing the selected nucleic acid sequences into at least one vector capable of directing expression of the selected nucleic acid sequences in an antigen-presenting cell.

2. The method of claim 1, wherein the antigen-presenting cell is a dendritic cell, macrophage, B cell, monocyte or fibrocyte.

3. The method of claim 1, wherein the vector further comprises an antigen-presenting cell targeting element.

4. The method of claim 1, wherein the first and second nucleic acid sequences are of the same tissue of origin.

5. The method of claim 1, wherein the selected nucleic acid sequences comprise at least 5 different nucleic acid sequences.

6. The method of claim 1, wherein the selected nucleic acid sequences comprise at least 7 different nucleic acid sequences.

7. The method of claim 1, wherein the selected nucleic acid sequences comprise at least 9 different nucleic acid sequences.

8. The method of claim 1, wherein the vector further comprises a nucleic acid sequence encoding an immunomodulatory cofactor.

9. The method of claim 8, wherein the immunomodulatory cofactor is IL-2, IL-3, IL-8, OKT3, α-interferon, γ-interferon, or MIP-1α.

10. The method of claim 1, wherein the vector further encodes at least one selectable marker.

11. The method of claim 10, wherein the selectable marker is PLAP, GFP or neomycin resistance.

12. The method of claim 1, wherein the target cell is a cancer cell.

13. The method of claim 1, wherein the target cell is a virus, a bacterium or a parasite.

14. A composition comprising at least one vector produced by the method of claim 1.

15. The composition of claim 14, wherein the vector further comprises an antigen-presenting cell targeting element.

16. The composition of claim 14, further comprising an antigen-presenting cell.

17. A method of producing an antigen-presenting cell that presents an array of antigens comprising:

(a) comparing first nucleic acid sequences expressed by a target cell population with second nucleic acid sequences expressed by a non-target cell population;

(b) selecting at least one nucleic acid sequence preferentially expressed by the target cell population relative to the non-target cell population; and

(c) genetically modifying an antigen-presenting cell to express the selected nucleic acid sequences.

18. The method of claim 17, wherein the antigen-presenting cell is a dendritic cell, macrophage, B cell, monocyte or fibrocyte.

19. The method of claim 17, wherein the first and second nucleic acid sequences are of the same tissue of origin.

20. The method of claim 17, wherein the selected nucleic acid sequences comprise at least 5 different nucleic acid sequences.

21. The method of claim 17, wherein the selected nucleic acid sequences comprise at least 7 different nucleic acid sequences.

22. The method of claim 17, wherein the selected nucleic acid sequences comprise at least 9 different nucleic acid sequences.

23. The method of claim 1, wherein the selected nucleic acid sequence further encodes at least one selectable marker.

24. The method of claim 23, wherein the selectable marker is PLAP, GFP or neomycin resistance.

25. The method of claim 17, wherein the target cell is a cancer cell.

26. The method of claim 17, wherein the target cell is a virus, a bacterium or a parasite.

27. An antigen-presenting cell produced by the method of any one of claims 17-26.

28. A method of activating T cells comprising contacting a T cell with an antigen-presenting cell of claim 27.

29. The method of claim 28, wherein the T cell is a cytotoxic T lymphocyte.

30. A method of inducing a toleragenic response comprising contacting a T cell with an antigen-presenting cell of claim 27.

31. The method of claim 30, wherein the T cell is a T_{H2} cell.

32. The method of claim 28 or 30, wherein the contacting occurs *in vivo*.

33. The method of claim 28 or 30, wherein the contacting occurs *ex vivo*.

34. The method of claim 32 or 33, wherein the activating is in the presence of an immunomodulatory cofactor.

35. The method of claim 34, wherein the immunomodulatory cofactor is IL-2, IL-3, IL-8, OKT3, α-interferon, γ-interferon, or MIP-1α.

36. A method of activating T cells *in vivo* comprising administering the composition of claim 14 to a subject.

37. A method of killing a target cell *in vivo* comprising administering the composition of claim 14 or the antigen-presenting cell of claim 27 to a subject.

38. A method of preventing infection comprising administering the composition of claim 14 or the antigen-presenting cell of claim 27 to a subject.

39. A method of treating cancer comprising administering to a subject the composition of claim 14 or the antigen-presenting cell of claim 27, wherein the target cell is a cancer cell.

40. A method of treating an infection comprising administering to a subject the composition of claim 14 or the antigen-presenting cell of claim 27, wherein the target cell is an infectious agent.

SUBSTITUTE SHEET (RULE 26)

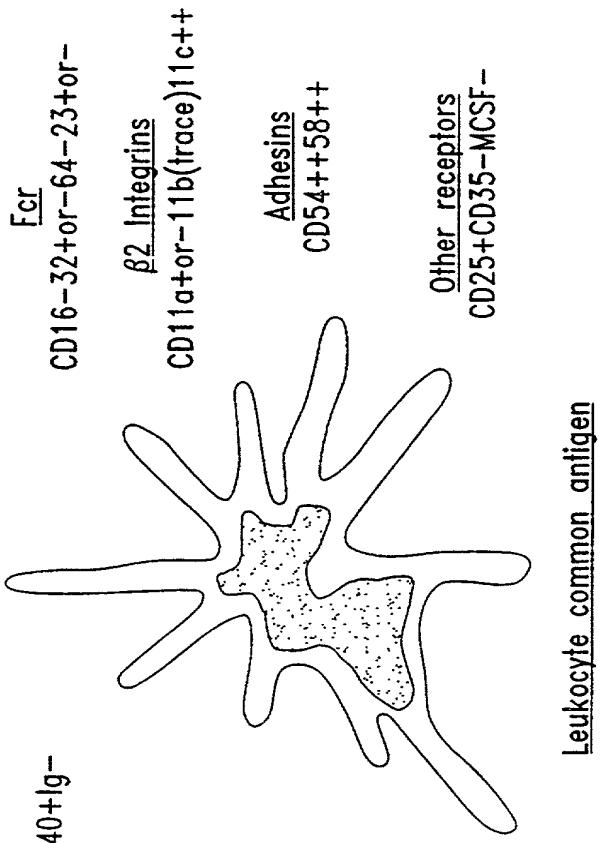
MHC
Class I++ Class II+++
B cell
CD19-29-21-22-40+Ig-
T cell
CD1+or-CD2-CD3-TCR-CD4+or-CD8+or-
Myeloid
CD13-14-15-36trace65-
NK cell
CD56-57-
Fcr
CD16-32+or-64-23+or-
β2 Integrins
CD11a+or-11b(trace)11c++
Adhesins
CD54++58++
Other receptors
CD25+CD35-MCSF-
Leukocyte common antigen
CD45+45RA-

Fig. 1

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Inventor's signature _____ Date _____

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Inventor's signature _____ Date _____

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Full name of third inventor Jaime Escobedo

Inventor's signature _____ Date _____

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400 Full name of fourth inventor Amy L. Collins

Inventor's signature Amy L. Collins Date 6/29/01

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Full name of fifth inventor Timothy Fong

Inventor's signature Timothy C. Fong Date May 18, 2001

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Inventor's signature Martin G. Giedlin Date 6/3/01

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Redacted - Confidential

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Full name of third inventor Jaime Escobedo

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